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Genetic Variants That Predispose to DNA Double-Strand Breaks in Lymphocytes From a Subset of Patients With Familial Colorectal Carcinomas

Sanjeevani Arora,[1,2] Hong Yan,[1] Iltaeg Cho,[3] Hua-Ying Fan,[3] Biao Luo,[4] Xiaowu Gai,[5] Dale L. Bodian,[6] Joseph G. Vockley,[6] Yan Zhou,[7] Elizabeth A. Handorf,[7] Brian L. Egleston,[2,7] Mark D. Andrake,[2] Emmanuelle Nicolas,[8] Ilya G. Serebriiskii,[2,9] Timothy J. Yen,[1] Michael J. Hall,[10,11] Erica A. Golemis,[2] and Greg H. Enders[1,12]

[1]*Cancer Biology Program,* [2]*Molecular Therapeutics Program,* [4]*Institute for Personalized Medicine,* [7]*Biostatistics,* [8]*Genomics,* [10]*Medical Oncology,* [11]*Clinical Genetics,* [12]*Medicine, Fox Chase Cancer Center, Philadelphia, Pennsylvania;* [3]*Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania;* [5]*Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts;* [6]*Inova Translational Medicine Institute, Inova Health System, Falls Church, Virginia;* [9]*Kazan Federal University, Kazan, Russia*

BACKGROUND & AIMS: DNA structural lesions are prevalent in sporadic colorectal cancer. Therefore, we proposed that gene variants that predispose to DNA double-strand breaks (DSBs) would be found in patients with familial colorectal carcinomas of an undefined genetic basis (UFCRC). **METHODS:** We collected primary T cells from 25 patients with UFCRC and matched patients without colorectal cancer (controls) and assayed for DSBs. We performed exome sequence analyses of germline DNA from 20 patients with UFCRC and 5 undiagnosed patients with polyposis. The prevalence of identified variants in genes linked to DNA integrity was compared with that of individuals without a family history of cancer. The effects of representative variants found to be associated with UFCRC was confirmed in functional assays with HCT116 cells. **RESULTS:** Primary T cells from most patients with UFCRC had increased levels of the DSB marker γ(phosphorylated)histone2AX (γH2AX) after treatment with DNA damaging agents, compared with T cells from controls ($P < .001$). Exome sequence analysis identified a mean 1.4 rare variants per patient that were predicted to disrupt functions of genes relevant to DSBs. Controls (from public databases) had a much lower frequency of variants in the same genes ($P < .001$). Knockdown of representative variant genes in HCT116 CRC cells increased γH2AX. A detailed analysis of immortalized patient-derived B cells that contained variants in the Werner syndrome, RecQ helicase-like gene (*WRN*, encoding T705I), and excision repair cross-complementation group 6 (*ERCC6*, encoding N180Y) showed reduced levels of these proteins and increased DSBs, compared with B cells from controls. This phenotype was rescued by exogenous expression of WRN or ERCC6. Direct analysis of the recombinant variant proteins confirmed defective enzymatic activities. **CONCLUSIONS:** These results provide evidence that defects in suppression of DSBs underlie some cases of UFCRC; these can be identified by assays of circulating lymphocytes. We specifically associated UFCRC with variants in *WRN* and *ERCC6* that reduce the capacity for repair of DNA DSBs. These observations could lead to a simple screening strategy for UFCRC, and provide insight into the pathogenic mechanisms of colorectal carcinogenesis.

Keywords: Colon Cancer; Hereditary Cancer; Genomic Instability; Tumorigenesis.

BASIC AND TRANSLATIONAL AT

Familial colorectal carcinoma (FCRC) is characterized by early disease onset and/or occurrence of CRC in multiple family members. Several FCRC syndromes have been linked with specific germline defects: familial adenomatous polyposis coli with the Wnt pathway gene *adenomatous polyposis coli*, Lynch syndrome with a group of mismatch repair genes (most commonly *MLH1*, *MSH2*, *MSH6*, and *PMS2*), and MutY-H polyposis with the eponymous base excision repair gene.[1] However, most FCRC remains genetically undefined (UFCRC), accounting for approximately 20% of CRC in the United States.

Clinical guidelines advise starting CRC screening in UFCRC families at earlier ages and, depending on family history, more frequent intervals.[2] Although beneficial, this strategy is inefficient. Family members who are not predisposed genetically are subjected to unnecessary costs and morbidity, although some of those actually at risk may be underscreened. Intensive genome-wide association studies have sought to identify additional genes underlying UFCRC. These studies have yielded only moderate associations at multiple genome locations, implying dauntingly complex genetics.[3–8] No common molecular defect has been recognized.

Abbreviations used in this paper: **BMP, bone morphogenetic protein; DSB, double-strand break; EVS, Exome Variant Server; ExAC, Exome Aggregation Consortium; FA, Fanconi's anemia; FCRC, familial colorectal cancer; HQV, high-quality variant; NER, nucleotide excision repair; PBL, peripheral blood lymphocytes; PP2, PolyPhen2; Pt1, patient 1; ROC, receiver operator characteristic; sCRC, sporadic colorectal cancer; siRNA, small interfering RNA; UFCRC, undefined familial colorectal cancer.**

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